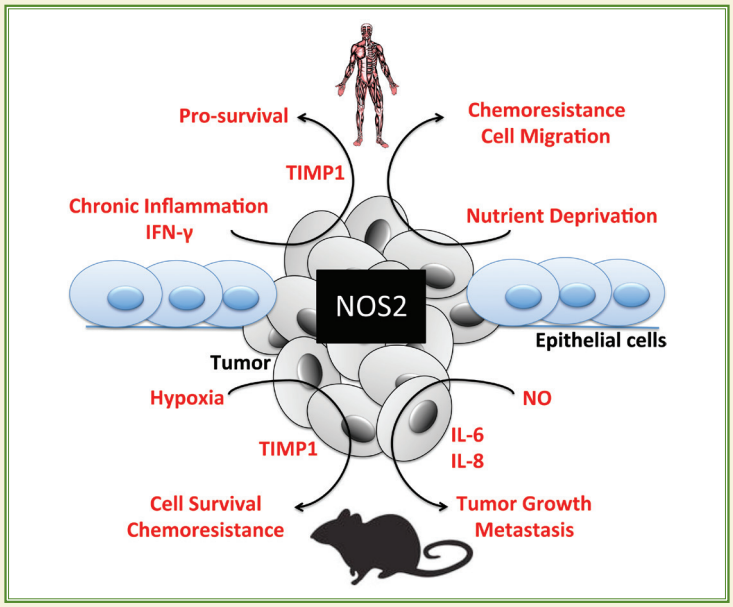
# From Expression to Action: the Answer is NO

*Nitric oxide plays an important role in aggressive breast cancers.*

Among the most aggressive breast cancers are those that lack the estrogen receptor α (ER-), and have a "basal-like" gene expression signature. In 2010, CCR researchers led by Stefan Ambs, Ph.D., M.Ph., Senior Investigator in the Laboratory of Human Carcinogenesis, in collaboration with a research team led by David Wink, Jr., Ph.D., Senior Investigator in CCR's Radiation Biology Branch, demonstrated that these ER- cancers frequently overexpress inducible nitric oxide synthase 2 (NOS2) which induces a basal-like gene expression pattern and also associates with poor patient survival. The researchers hypothesized that NOS2 may play an important causal role in the progression of ER- cancers. A new study, published in *Proceedings of the National Academy of Sciences*, set out to further validate this observation, and to identify the likely mechanisms of its action.




(Image: J. Heinecke and L. Ridnour, CCR)

Nitric oxide synthase (NOS2) participates in an extensive network driving cancer progression and metastasis in ER- breast cancer.

Establishing causality in cancer is not the work of a single study, approach, or laboratory, so a multidisciplinary team led by Wink including Ambs, as well as researchers from the Pediatric Oncology Branch and the Laboratory of Experimental Immunology, took up the challenge. Their strategy included two broad approaches: (1) manipulating nitric oxide (NO) levels in cell and animal models with the NOS2 inhibitor aminoguanidine (AG), and the NO-donor DETA/NO, and (2) simulating the tumor microenvironment by placing cells in the context of serum withdrawal, hypoxia, cytokines, and cancer therapeutics.

The team chose an aggressive mouse model of breast cancer: MDA-MB-231 human breast cancer cells implanted in the mammary tissue of female mice with compromised immune systems. They tagged the cells with green fluorescent protein (GFP) to make them easy to identify. After 40 days, the reduction in tumor volume and metastases to the brain was dramatic in those mice treated with the NOS2 inhibitor. Furthermore, expression of genes associated with the ER- basal-like signature, *IL-8, IL-6, S100A8, CD44,* and *TLR4,* were all reduced in the treated mice.

To simulate the tumor microenvironment, in which cells become packed together, without proper exposure to oxygen and other circulating factors, the researchers grew breast cancer cells in culture and deprived them of serum. Serum withdrawal normally induces cell migration, but NOS2 inhibition detained the cells, an effect that could be overcome with an NO donor. NOS2 inhibitors also prevented the development of resistance to the chemotherapeutic taxol in this model.

If NOS2 plays a substantive role in tumor growth, migration, and chemoresistance, what is inducing it? As expected, they found that both hypoxia and serum withdrawal caused a strong increase in NOS2 mRNA expression in breast cancer cells, as did a mix of cytokines, interferon-γ, and inhibition of NOS2 itself, suggesting the potential for a positive feedback mechanism that furthers the aggressiveness of the cancer.

"Understanding the factors within the tumor microenvironment that regulate NOS2 and NO flux-driven tumor progression could lead to more personalized therapeutic options for women whose breast tumors express high NOS2," said Wink. "The use of NOS2 inhibitors combined with inhibition of upstream and downstream targets could improve clinical outcomes."

*To learn more about Dr. Wink's research, please visit his CCR website at https://ccr.cancer.gov/david-a-wink.*